The Study on the Patency of the Perforating Arteries after Stent Placement in Atherosclerosis Induced Rabbits

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Summary

The number of successful case reports with percutaneous transluminal angioplasty (PTA) / stenting for intracranial atherosclerotic stenoses is recently increasing with the advent of flexible coronary stents. However, it is not well known whether the perforating artery is occluded or not after stent placement in the atherosclerotic stenotic vessels. We investigated this issue using five New Zealand white rabbits. We deployed stainless steel stents in the atherosclerosis-induced abdominal aorta across the lumbar artery in which the diameters of the abdominal arteries were similar to those of human intracranial arteries. We evaluated the patency of lumbar artery by angiography and scanning electron microscopy three months after stent placement. The lumbar arteries were patent in four out of five rabbits. However, SEM findings demonstrated stent struts were covered with thick neointima and the ostia between stent struts were partially occluded. It is possible that stent placement in the atherosclerotic arteries can cause the obliteration of the perforating arteries.

Introduction

The flexible stents has recently become available for intracranial arteries, such as stent supported coil embolization for broad neck aneurysm [1,2] and stenting for atherosclerotic stenosis [3-7]. In stent supported coil embolization for broad neck aneurysms, stents are usually placed in normal, non-stenotic arteries. We previously reported that stent placement in normal arteries could keep the patency of the perforating arteries, even if the stent struts covered the ostium of the perforating arteries [8]. However, the neointimal response after stent placement in the atherosclerotic arteries is thought to be quite different from that of the normal arteries. Thus, our major concern related to intracranial stenting for atherosclerotic stenoses is the patency of the perforating arteries after stent placement. In this study, we investigated this issue using atherosclerosis-induced rabbits.

Material and Methods

We have already reported that the relationship between the abdominal aorta and the lumbar arteries in the rabbits is anatomically similar to that between the major intracranial artery and perforating arteries in humans with regard to the diameter and branching angle.

Thus, we performed stent placement in the abdominal aorta across the ostium of the lumbar artery in the atherosclerosis-induced rabbits, and evaluated the patency of the lumbar arteries using angiographic and scanning elec-

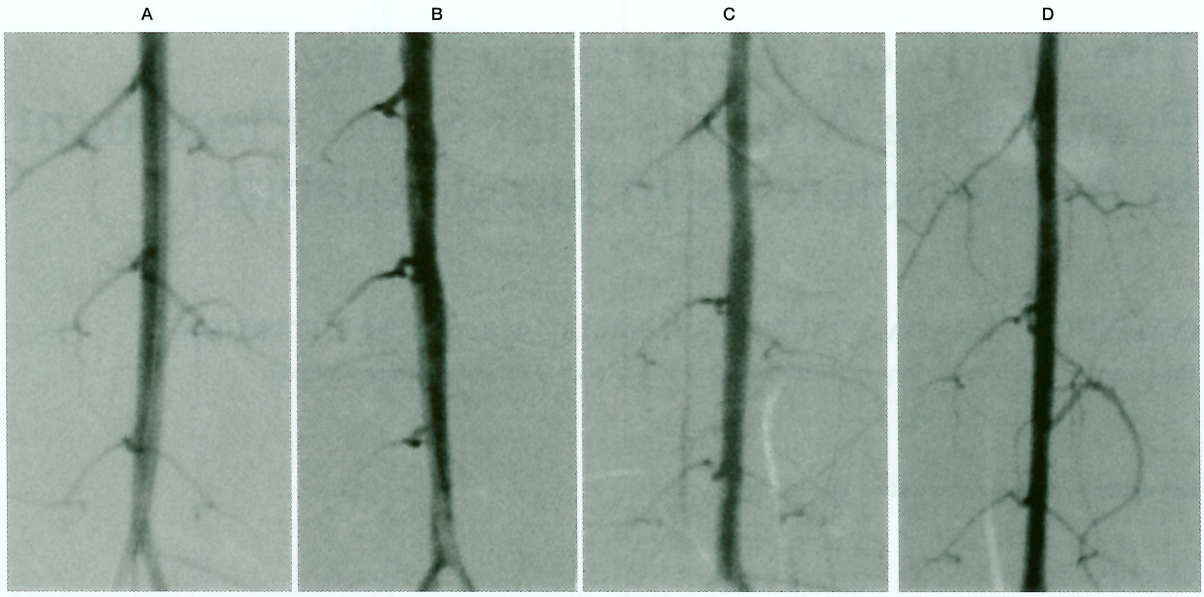

Figure 1 Angiographic changes of the representative case. A): post-denudation, B) pre-stenting 6 weeks afler denudation), C) just after stenting, D) follow-up (three months after stenting).

tron microscopic and histopathological examinations.

Materials

New Zealand white rabbits were used in this experiment, because the induction of atherosclerosis had been established[9]. All animal care conformed to the institutional guidelines of Wakayama Medical University.

Induction of Atherosclerosis

Five New Zealand white rabbits weighing 2 to 3 kg, were initially fed hypercholesterol diet (2% cholesterol, 3% coconut oil) for 4 weeks. After that, these rabbits underwent balloon denudation of their abdominal aorta. Briefly, they were anesthetized by intravenous injection with pentobarbital (4 mg/kg).

Each right femoral artery was exposed and a 4-French sheath was inserted by arteriotomy of the femoral artery. After angiography as a control, the endothelium of the abdominal aorta was denuded by withdrawing the inflated 3-French Fogarty balloon catheter (Baxter) three times.

After post-procedural angiography, the surgical procedure was finished. Just before balloon denudation, heparin (100U/kg) was given intravenously. Aspirin (5 mg/kg) was administered orally from one day before the surgery to 1 month after stent placement. All rabbits were fed hypercholesterol diet continuously after balloon denudation for six weeks.

Stent Placement

These atherosclerosis-induced rabbits underwent follow-up angiography via the left femoral artery by the same procedure of balloon denudation. At first, stenotic ratio was measured using measure wire. The stenotic ratio was defined as the ratio of the diameter of the pre-stenting vessel divided by that of the pre-denudated vessel. The stainless steel balloon expandable stent (MULTI-LINK™, Guidant/Advanced Cardiovascular Systems Inc., Temecula, CA) was deployed at the most severe stenotic abdominal aorta across the orifice of the lumbar artery just after intravenous administration of heparin (100U/kg). Each stent was deployed at 6-8 atm. for 15 seconds. After post-stenting angiography, the surgical procedure was finished.

Follow-up Angiography

Follow-up angiography was performed via the left common carotid artery three months after stent placement to evaluate the patency of the lumbar artery.

Scanning Electron Microscopy

After the follow-up angiography, four of five rabbits were euthanized for scanning electron scanning (SEM) analysis as reported previously. Inferior vena cava exsanguination was performed with perfusion of 0.2M phosphate buffer salme (PBS) with heparin via the left ventricular puncture. The stented arteries were then harvested and fixed with 0.1M PBS, including 1% paraformaldehyde and 1.25% glutaraldehyde. After rinsing with 0.1M PBS, we gently opened the stented arteries with tungsten scissors not to damage the intraluminal surface, and these specimens were post-fixed with 1% OsO4 under 4°C for 1 hour. The stented arteries were then dehydrated with graded ethanol and t-butyl alcohol, and freeze-dried. After coating with 20 nm of gold, we examined the patency as well as the neointimal thickness at the ostia of the lumbar arteries.

Histopathological Evaluation

One rabbit's stented aorta was evaluated for histopathological analysis at the ostium of the lumbar artery using histopathological methods. After exsanguination, the stented artery was harvested and then fixed with 10% neutral buffered formalin. The stented artery was carefully cut with a tungsten knife at 4-5 μm thickness. The cut sections were stained with hematoxylin-eosin and Masson-Trichrome to examine the cellular composition.

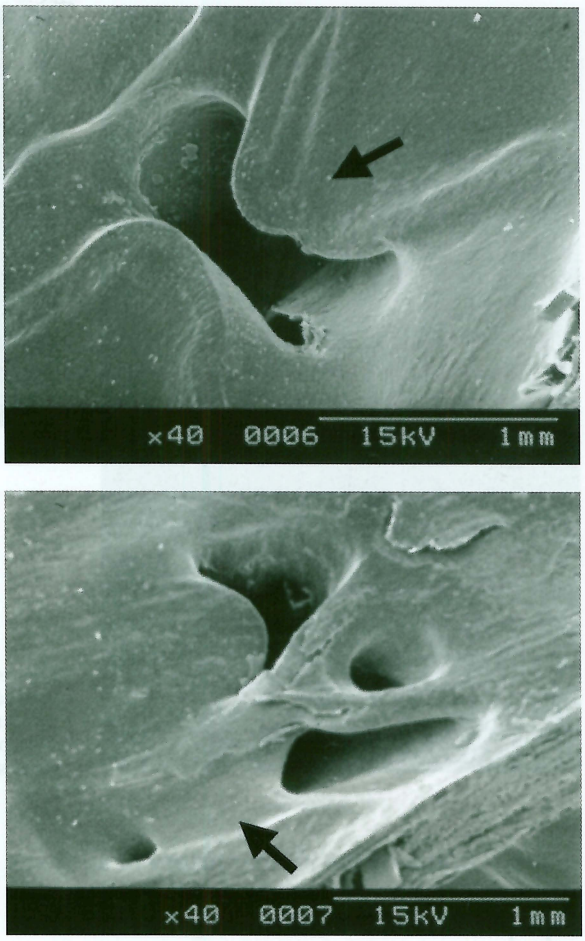

Figure 2 Representative SEM findmgs A) N° l, B) N° 2 rabbit. The stent struts crossing the orifice of the lumbar artery are covered with thick neointima. Arrows indicate stenosis of the orifice.

Results

Atherosclerosis-induced Aorta

Six weeks after balloon denudation, the arterial wall of the denuded aorta had macroscopically become thicker. Microscopic findings revealed neointimal hyperplasia with disruption of the internal elastic lamina. The neointima consisted mainly of hypertrophic smooth muscle cells and fibrous tissue.

The denuded aorta demonstrated atherosclerotic changes with segmental stenotic lesions on angiogram.

Angiographic Findings of the Stented Aorta

The angiographic results were summarized in table 1. The average stenotic ratio at the most severe stenotic abdominal aorta involving lumbar artery was 25%. The lumbar arteries were kept patent without late filling in all cases on angiogram before and just after stent placement. The follow-up angiogram demonstrated that the lumbar arteries in four of five rabbits were still open without late filling. However, the lumbar artery in one rabbit was completely occluded with intraluminal thrombus. Restenosis in stented aorta was not encountered on the follow-up angiogram. The angiographic changes of representative case were shown in figure 1.

Scanning Electron Microscopic Findings

SEM was performed in four rabbits to investigate the pathomorphologic status of the ostium of the lumbar artery. The stent strut cros-

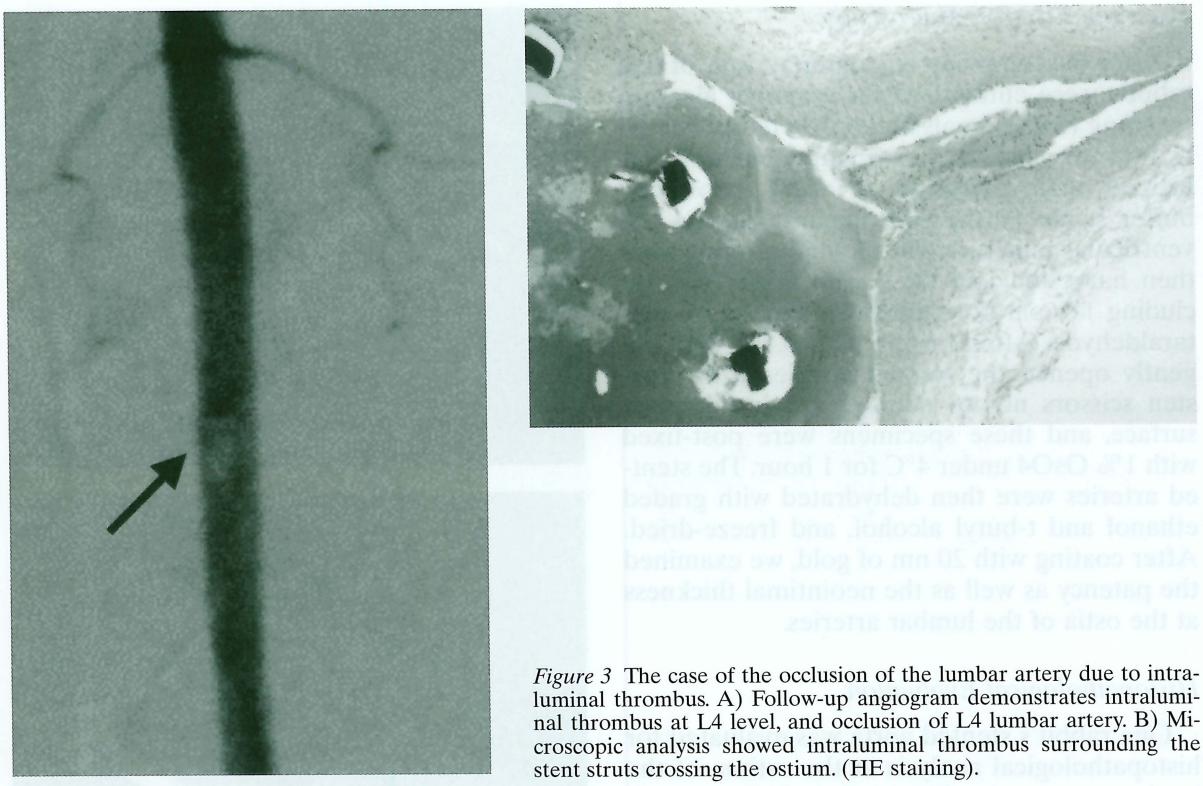

Figure 3 The case of the occlusion of the lumbar artery due to intraluminal thrombus. A) Follow-up angiogram demonstrates intraluminal thrombus at L4 level, and occlusion of L4 lumbar artery. B) Microscopic analysis showed intraluminal thrombus surrounding the stent struts crossing the ostium. (HE staining).

sed the ostium of the lumbar artery in all cases. Although the ostium of the lumbar artery was kept patent, luminal narrowing was shown in all cases.

The stent strut was completely covered with the thick neointima. Besides, the small ostial space enclosed by the stent strut led to the stenosis due to the proliferated neointima in three rabbits. Representative SEM findings were shown in figure 2 (A: N° 1, B: N° 2).

Histopathologic Findings

In the macroscopic findings of the stented arteries, the stent struts crossing the otstium were covered with a thick neointima, although os-

Table 1 **Results of Experimental Cases**

			Status of the orifice three months after stenting	
Case No	**Stenosis Ratio***	**Re-stenosis of stented vessel**	**Angiographic findings**	**SEM findings**
1	20%	(-)	late filling (-)	luminal narrowing (+)
2	30%	(-)	late filling (-)	luminal narrowing (+)
3	25%	(-)	late filling (-)	luminal narrowing (+)
4	20%	(-)	late filling (-)	luminal narrowing (+)
5	30%	(-)	occlusion	

** Stenotic ratio = pre-stenting vessel diameter/pre-treatment vessel diameter*

tium of thee lumbar artery was patent. Microscopic analysis of stented arteries demonstrated that stent struts were covered with the thick neointima. This neointima was composed mainly of the proliferated smooth muscle cells. The neointima of stented atherosclerotic vessels was obviously thick compared with that of normal vessels.

In case of occlusion of the lumbar artery due to the intraluminal thrombus on follow-up angiogram, the thrombus existed surrounding the stent struts crossing the ostium of lumbar artery. The thrombus extended into the lumbar artery. This thrombus was thought not to be formed just after stenting, because the infiltration of macrophage without fibrous changes was shown in the thrombus (figure 3).

Discussion

The number of cases of successful intracranial stenting has recently been increasing [1-7]. However, the use of stents for intracranial arteries has not been established yet, because intracranial arteries have specific characteristics. One of them is that they have many perforating arteries.

We previously reported that these perforating arteries were kept patent after stenting if the parent artery is normal [8]. However, it is well known that the atherosclerotic vessels have different characteristics, such as defect of endothelial cells, and hyperactivity of smooth muscle cells, compared with the normal vessels. Thus, we speculated that different response could be shown in the perforating arteries of atherosclerotic vessels after stenting. In coronary arteries, side branch occlusion after stenting has also been one of the interesting concerns from the beginning of coronary stenting. Several clinical reports about side branch occlusion have already been published [11-13]. Poerner et Al. reported whether small and medium-sized side branches could occlude or not after stenting for the coronary arteries. On their clinical study, the side branch occlusion occurred in 21.2% and had a benign course.

The most affected factor for side branch occlusion was the diameter of the side branch (less than 1.4 mm). On the other hand, perforating arteries occlusion could cause significant neurological deficits although diameter of perforating arteries was much smaller than that of the coronary side branch. There are no clinical or experimental reports about the changes in perforating arteries after stenting for the intracranial major arteries. In this study, we used the rabbits' abdominal aorta and the lumbar artery to resolve this problem.

Because the relationship between major intracranial arteries and perforating arteries originating from these arteries in humans approximated that of the abdominal aorta and lumbar arteries in the rabbit with respect to the diameter and the angle of branching as we described previously. In our current study, the response of experimental atherosclerosis-induced vessels after stenting was quite different from that of non-atherosclerotic vessels. In previous our experiment, the ostium of the lumbar arteries was widely open in all cases. While, in this study, the stent struts crossing the ostium of lumbar arteries were covered with the thick neointima. SEM findings showed the ostial stenosis. In one case, the lumbar artery was completely occluded due to the intraluminal thrombus. It is unclear whether in-stent thrombosis is a specific complication for atherosclerotic vessel or not. But there is possibility that intracranial stenting for atherosclerotic stenosis could cause occlusion of the perforating arteries. In this current study, the small ostial space enclosed by the stent strut tended to be stenosed. According to our results, the stents, composed of thinner struts may be effective to prevent occlusion of the perforating arteries. Recently the more suitable stents have been developed, such as drugeluting stents to inhibit the neointimal growth.

Marix et Al. reported that sirolimus-coated stents could completely control the proliferation of smooth muscle cells [14]. Intracranial stenting can be established as one of the treatments for revascularization of the brain, if these optional stents can resolve the problem of perforating arteries occlusion as well as restenosis.

Conclusions

Intracranial stenting for atherosclerotic stenosis has a possibility to cause the occlusion of the perforating arteries.

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